# MULTIPLE ANTIGEN PEPTIDE SYSTEM HAVING ADJUVANT PROPERTIES, VACCINES PREPARED THEREFROM AND METHODS OF USE THEREOF

The present invention was made with partial assistance from Grant No. A128701. The Government may have certain rights in the invention.

## CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of application Ser. No. 07/877,613, filed May 1, 1992, which is now abandoned.

## BACKGROUND OF THE INVENTION

The present invention relates generally to the field of immunology, and particularly to the preparation and administration of vaccines for prevention and treatment disease states such as HIV infection.

Highly specific and immunogenic antigens are preferred as vaccines. While the immunogenicity of an antigen can be increased by coupling a protein carrier to the antigen, this approach has several drawbacks. First, if the carrier is large, significant humoral immune response can be directed against the carrier rather than the antigen. Second, a large carrier can suppress humoral response to the antigen. Finally, the coupling of an antigen to a protein carrier can alter the immunogenic determinants of the antigen.

Multiple antigen peptide systems (MAPS) are designed to overcome the problems observed with conventional protein careers. Most MAPS are composed of several peptide antigens covalently linked to a branching, dendritic core composed of bifunctional units (e.g., lysines). Thus, a cluster of antigenic epitopes form the surface of a MAPS and a small matrix forms its core. As a result, the core is not immunogenic. MAPS have been used to prepare experimental vaccines against hepatitis (Tam et at., *Proc. Natl. Acad. Sci. USA* 86: 9084, 1989), malaria (Tam et at., *J. Exp. Med.* 171: 299, 1990), and foot-and-mouth disease. A further advantage of MAPS is that they are chemically unambiguous. This allows different epitopes, such as B cell and T cell epitopes, to be arranged in a particular arrangement and stoichiometry.

Specific MAPS have been and are in development for use in immunization against HIV. For example, European Patent Publication No. 0 339 695 published 11 Feb. 1989 describes a process for preparing MAPS by reacting a branched structure based on an amino acid such as lysine or ornithine with a separately synthesized antigenic compound.

European Patent Publication No. 0 328 403, published 16 Aug. 1989 describes particular peptides that are specifically immunoreactive with antibodies to HIV and suggests that MAPS which include these peptides can be used for immunization to prevent HIV infection.

Hart et al. (*J. Immunol.*, 145: 2677, 1990) report that a synthetic peptide construct which includes amino acids 428–443 and 303–321 of HIV-I-III, envelope protein gp120, when used as a carrier-free immunogen in primates, can induce a high titer of neutralizing anti-HIV antibodies and can induce T cell proliferative response against native HIV-1 gp120.

Palker et al. (*Immunology* 142: 3612, 1989) describes the use of a 16 amino acid T cell epitope from HIV-1-IIIB fused to a synthetic peptide which includes a type-specific neutralizing determinant of a particular HIV-1 strain (III. 1 MN or RF) to immunize goats. Both T cells and B cells responded to epitopes within the type-specific neutralizing determinant.



PCT Application Publication No. WO 90/11778 published 18 Oct. 1990 discloses multiple antigen peptide systems in which a large number of each of T cell and B cell malarial antigens are bound to the functional groups of a dendritic core molecule.

In Copending U.S. application Ser. No. 07/744,281, now abandoned, by Tam et al., a particular multiple antigen peptide is prepared for use as a vaccine for the treatment of HIV infection that incorporates particular peptides derived from the HIV-1 $III_B$ envelope protein as well as the V3 loop of the gp120 protein of HIV 1-MN. This peptide system demonstrates the capability of generating a humoral response and the development of antibodies, and seeks to elicit a T cell response by the inclusion of a T cell epitope. The in vivo administration of this peptide requires the inclusion of an adjuvant as a means of enhancing the humoral response.

More generally, most vaccine strategy developed today particularly against human immunodeficiency virus (HIV) infection has been directed toward the humoral response of generating neutralizing antibodies. Recent advances in mapping antigens involved in immune responses have allowed detailed characterization of epitopes that confer neutralizing, T-helper and T-cytotoxic responses. These developments have led to consideration of including the T-cytotoxic response along with humoral immunity in the design of peptide-based vaccines.

As noted above and elsewhere, traditional methods for preparing peptide vaccines that present peptides as macromolecules through conjugation to protein carriers or polymerization are often unable to induce cytotoxic T lymphocytes (CTL) response in vivo. Use of an adjuvant in the immunizing protocol has the advantage of enhancing the humoral response but has mixed results in priming specific CTL response. Furthermore, the most popular adjuvant used in laboratory animals, such as Freund's complete adjuvant, is too toxic and unacceptable for humans. Ideally, protection against viral infection is best provided by both humoral and cell-mediated immunities, including long-term memory and cytotoxic T cells.

Specifically, the human immunodeficiency virus (HIV), the etiologic agent of the acquired immunodeficiency syndrom (AIDS), has become an important objective for various vaccine developments. The predominant vaccine strategy has focused on the use of the envelope protein antigens gp120 and gp160 of HIV-1 produced by the recombinant DNA technology. However, the full promise of their use in vaccines will not be realized unless they are administered along with an effective adjuvant.

An adjuvant is usually a non-toxic agent that provokes specific responses to antigens. There is a wide spectrum of mechanisms by which an adjuvant functions. It can function by creating a depot at the site of injection that prolongs the release of antigens with antigen-presenting cells. It may also function by activating macrophages to release cytokines and mediators which in turn activate effector T cells or antibody-forming B cells. The net result is that an adjuvant augments specific humoral and cell-mediated immunities with a lower dose of antigen required.

Many seemingly unrelated agents have been used as adjuvants and the commonly used adjuvants can be broadly categorized into four groups. The first, and the only clini-